Marches régulaires #7
le 10.03.2003

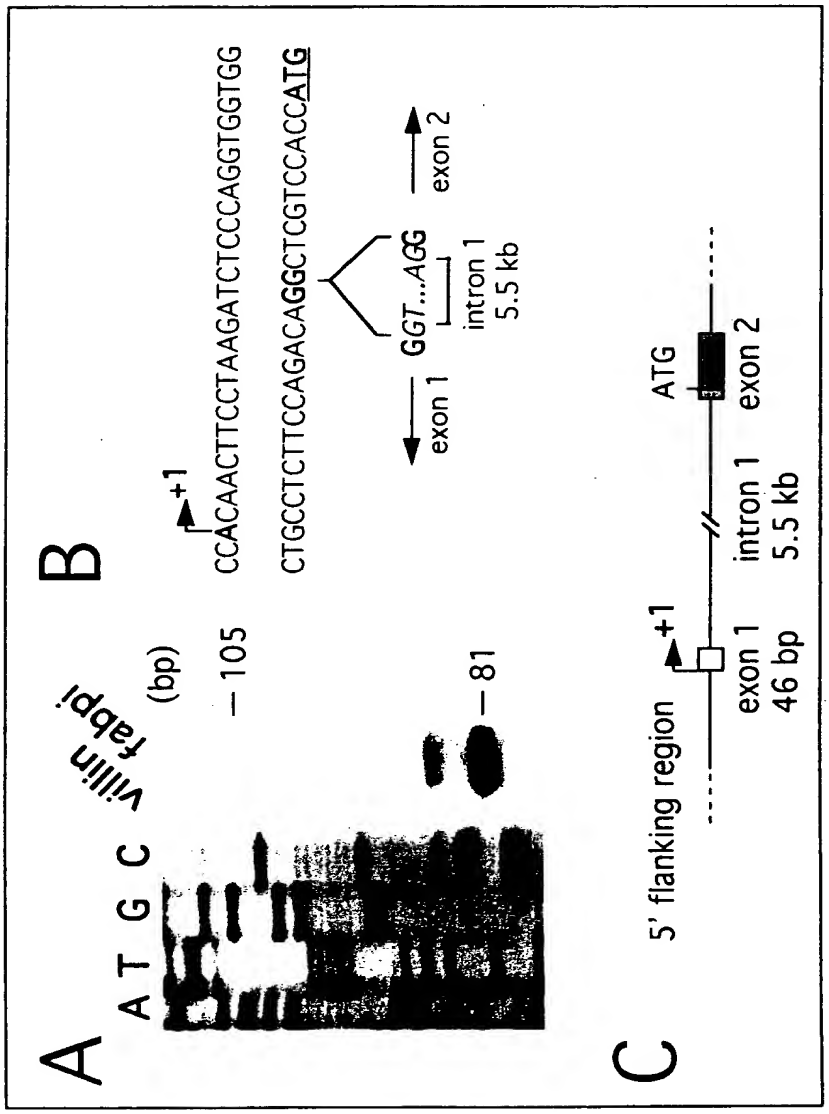


Figure 1

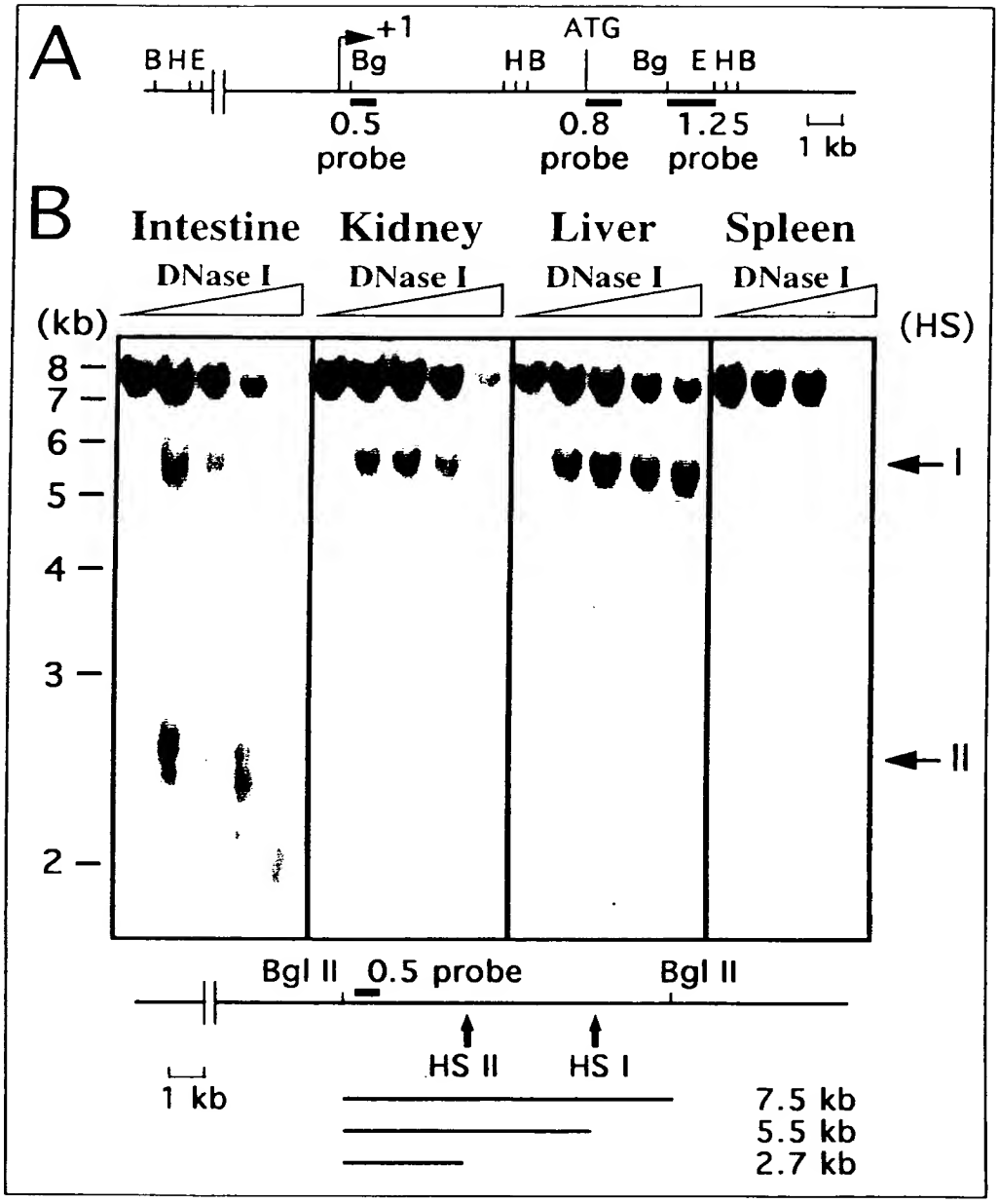

A
BHE
+1
Bg
HB
ATG
Bg EHB
0.5 probe
0.8 probe
1.25 probe
1 kb
B
Intestine
Kidney
Liver
Spleen
DNase I
(kb)
(HS)
8
7
6
5
4
3
2
I
II
Bgl II
0.5 probe
Bgl II
HS II
HS I
1 kb
7.5 kb
5.5 kb
2.7 kb

Figure 2 (a)

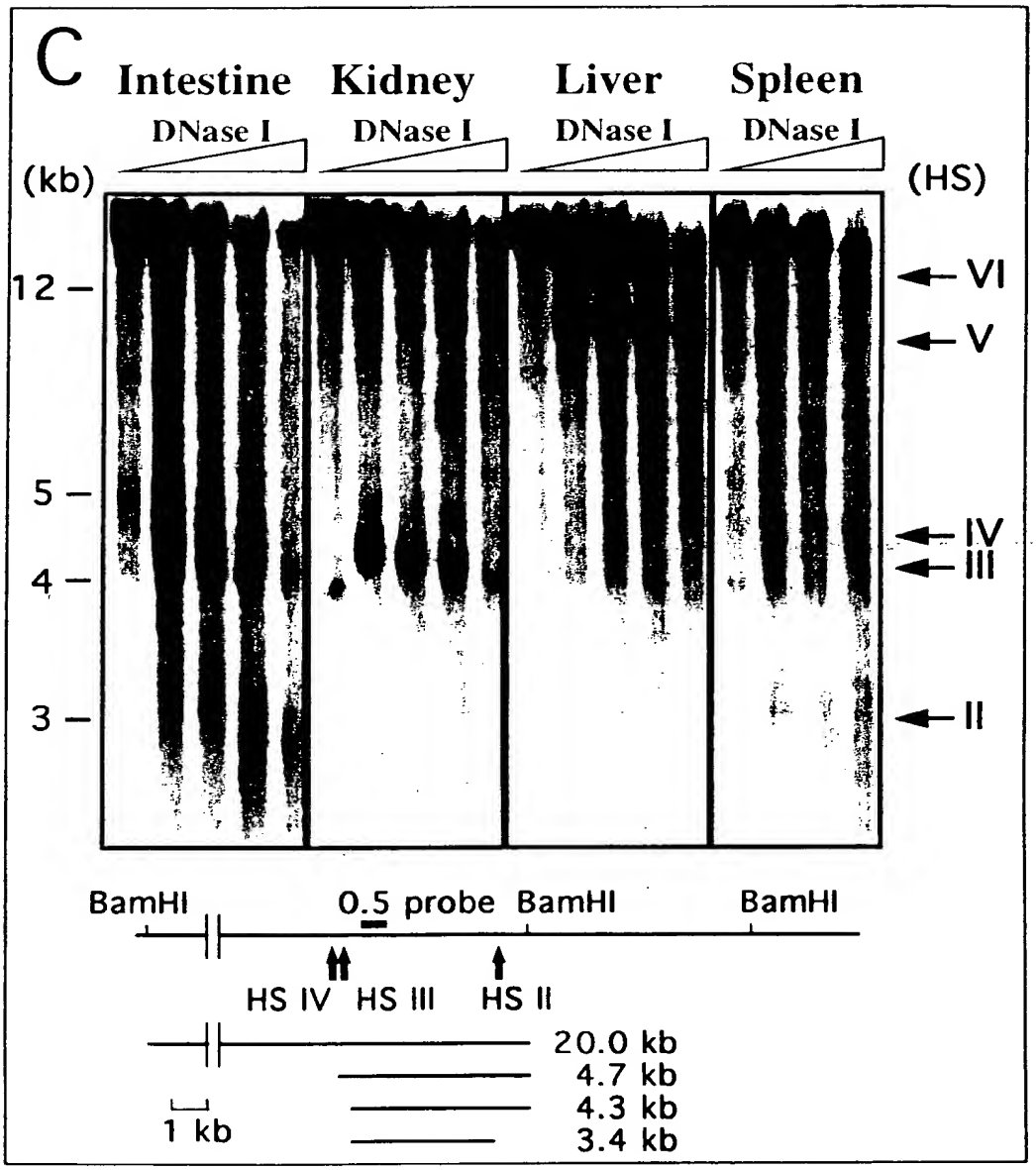
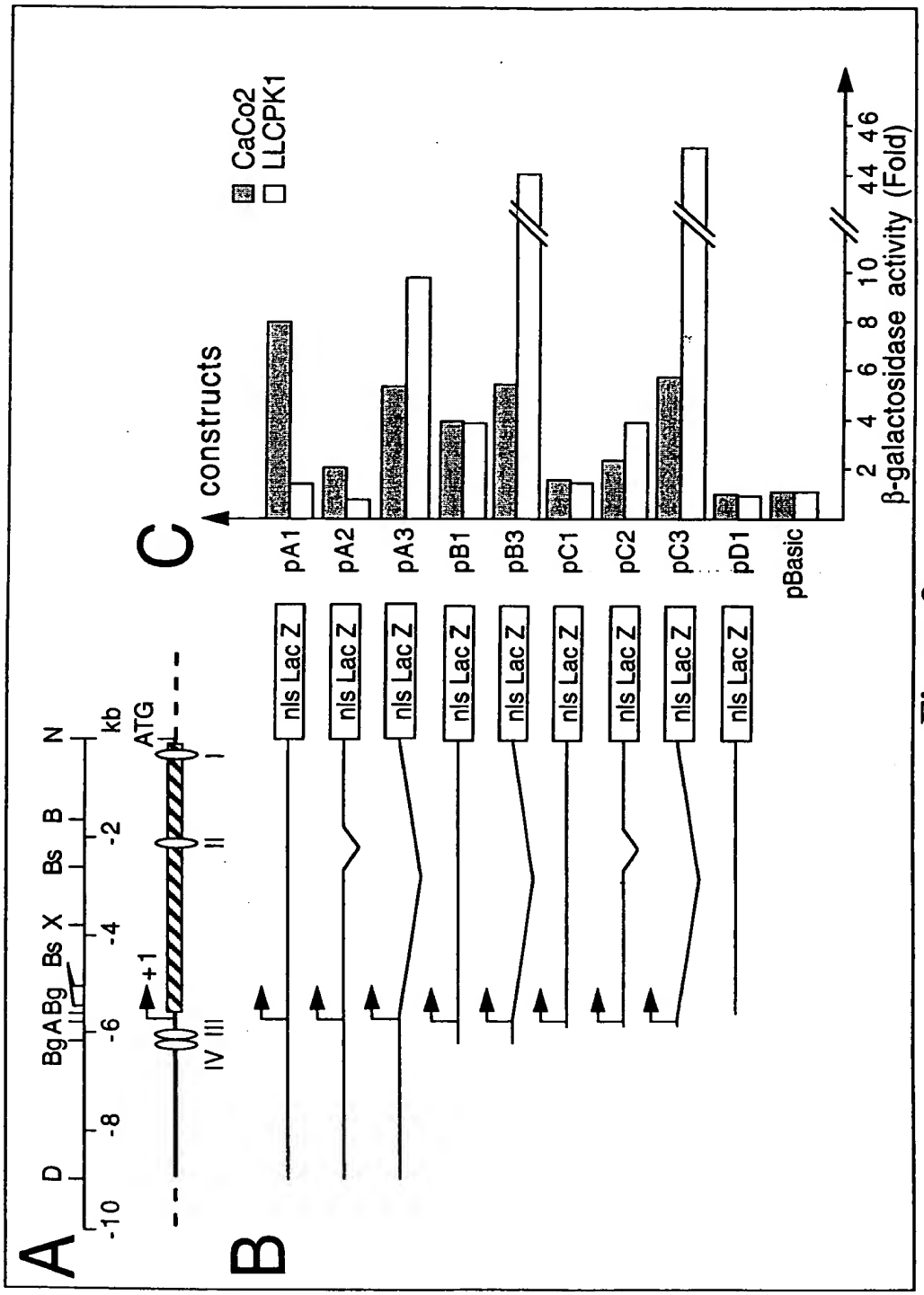

C
Intestine
Kidney
Liver
Spleen
DNase I
DNase I
DNase I
DNase I
(kb)
(HS)
12
5
4
3
VI
V
IV
III
II
BamHI
0.5 probe
BamHI
BamHI
HS IV
HS III
HS II
20.0 kb
4.7 kb
4.3 kb
3.4 kb
1 kb

Figure 2 (b)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D
-10
-8
Bg
A
Bg
Bs
X
-6
-4
+1
Bs
B
-2
kb
N
ATG
IV
III
II
I
B
nls Lac Z
C
constructs
CaCo2
LLCPK1
pA1
pA2
pA3
pB1
pB3
pC1
pC2
pC3
pD1
pBasic
2
4
6
8
10
44
46
β-galactosidase activity (Fold)

Figure 3

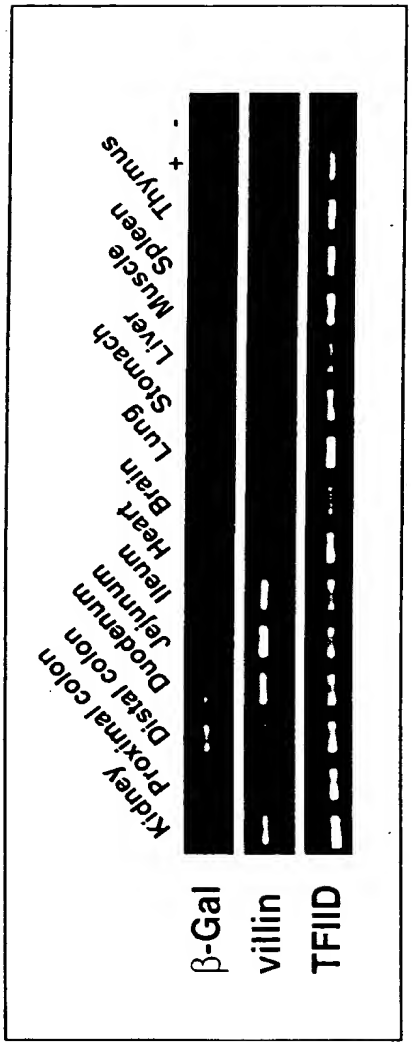
β-Gal
villin
TFIID
Kidney
Proximal colon
Distal colon
Duodenum
Jejunum
Ileum
Heart
Brain
Lung
Stomach
Liver
Muscle
Spleen
Thymus
+
-

Figure 4

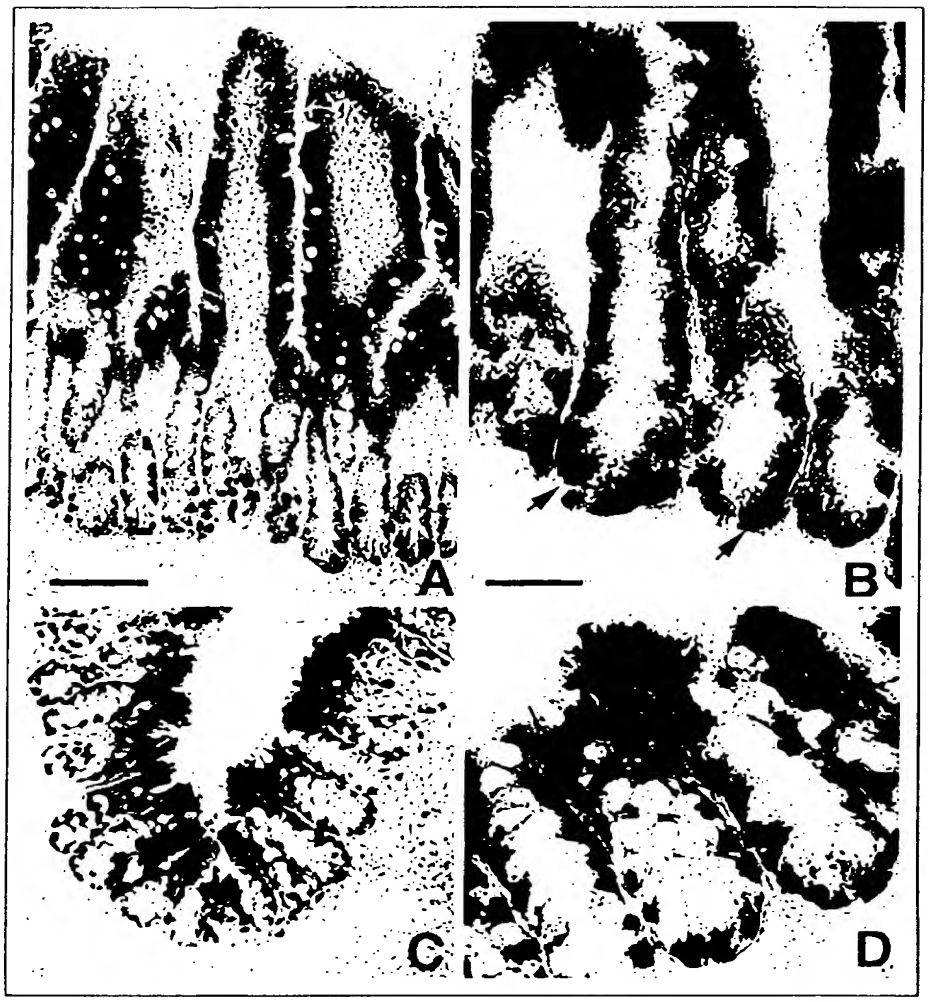

Figure 5

Gen mic sequenc f the m use villin g n r gulat ry sequences

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CCTTTGTTTG TTTGTTTGTT TGTTTTAAAG CATTAATAAA TAATACCATA AGGAGGTTGG 180
CAGTGGTGGC AGACACCTTT AATTCCAGTA TTCAGGAGGC AGAAGCAGGC AGATCTCTGT 240
GAGTTCGAAG TCAGCCTAGT CTGCAAAGCT AGTTCCAGGA TGGCAAGGGC TACACAGAGA 300
AACCTTGTCT CATAAAACCA AAGTAGTAGT AGTAGTAGTA ATGCCATAGA GAAAATTGGA 360
GTCCATTCAG GATGGACCAT CCTATAAGAT GATTCTCTTG ACCCAGGTAA GCTAATGTCA 420
TGGGGAAAGG GGATGGGACT GTCCTAGATT AAAAAGTGCT GAGGCGATGC CTATTCTCAA 480
TTTGATTCCA TATGAAAAGG CTGATAAGGC CCAAGAGAAG TGGAACTGGG ACTCTGGACT 540
GAAGACGTGA CGGCCTTATA AACACTGGCA CTTATAAACA CTTATAAACA CTGGCACAGG 600
CGTTCAGGTT TGAAGATCAC TTTCAAACCA CAGAACAGAA AGTGCTCGCT CGTCCTCAGC 660
GTAGCGAGCA CTGGCTGCAG AAGAGTGATA TTTAGTGAAA GCTACCTTCA CAATATCTTT 720
GCACTTATCA CATACACGTG TCAAATGTGC TAACTCCCTA GTCCACAGAT GGCTGTTACA 780
CTCGTTTCTG CTTTCCCATC TGGTTGACAT TTGTCAGAAC CAGAAATTAG AAATGTGGGT 840
ATTTATTTGT GTGCTGAGGA CACCATCCAG GGCTTTTCAC ATTTCAGGCA CATGGTTTAC 900
TAACTGGGCT ACTTCTCCAA CGGTTTGAAA CCATTTGTTT TATATTTACT TATTTTGTGT 960
GCATGAGGTA GGCATGTATA CGTATGTATA GGAGTCATGC ATGTGGCTGC TACCCTCAAA 1020
ATCATTGCAG ATCCCCAGCA AGTGAAGTCA CCGAGCGTTG TAAGTTGTTA TGTGGGACTG 1080
GGAGCCAAGG CTGGGTTCTC TGCAAGAGCA GCCAGTGGCC TTAACCATGG GACCAGCTCT 1140
CTAGGCCTAA GGTAATCTTT AGTTTTTTAA AAATATATAT TCTCAGCCGG GTGTGGTGGC 1200
ACACGCCTTT AATCCCAGCA CTTGAGAGGC TGAGGTGTAG GAATTATACA CACAGGCCAG 1260
CTGGGGTGCA GAGCTTGGCC CTGTTTTTTT TGTTTTTTCT TTATGTGCAC TGGTGTCTTA 1320
CCTGCGTGTA TGTCCGTGCA AGGGTGTCAG ATCCCTTGGA GCTGGAGTTA AAGACAGTTG 1380
TGATCACGCT GCCGTTACAG ATGCTGGAAA TTGAACCCAG GTGTCCCTAG AGAAGCAGCC 1440
AGTGCTCTTA ACTTCTGAGC CACCCCTCCA ACCCTGCTTT TAGAGACTCT TAACCTTTTG 1500
TGTAATGTGG GAACTGAGTG GATCTTGCAC TTACCAAGTG TGTGCTGCGC TGTAGCATCA 1560
CTGAGCCCGT ACCCACACGA CTAGTGGATA CAGTTTAAGG GCAAACACTT AACAATGACA 1620
ATAGTTGGAT AGAGTTTGAA TATAGTCCTG AGCTATTGGT TAGCGTGACC TTTGCTGTCC 1680
TTAGCATGTG CTGTGAGAAG ATAGAAAAAT GAAGACTTGA GTCTAGTCCT GGAACCCACA 1740
GAGGCAGGCG AGAACCCACT CCTGAAAGTT GTTCTCTGAG CTTCACATAC AACTTCACAT 1800
```

FIGURE 6A

AATAGTTACA ATGATAATAA TAATTAGTAA ATTCTTTTAA AAGGTATATG TTGGGAGGGA 1860
GAGATGGCTC AGCTTCCAGG AGCACTTGCT GCTCTTGCAG AGGACCTAGA TTCAGTTCCC 1920
AGGACTCATA TGGTGGCTCA CAGCCATCTG TAAATCCAGT TCCAGAGGGT TCCACACCCT 1980
CTTCTGGCCT CCACAGGCAC CACATACATA GTACACAGAC ATACATGCAG GCAAAACACC 2040
CATACACACA TAAATAAATA AGGAAACTTA AAAGGTGCAT GTGTTGGTAA ACATTGTGCT 2100
TACACATGCT GATTGAAGAC ATGTACAACG CACACACTGA AGAGGGATCT GGGGCTGGAG 2160
AGATGGCTCA GCGGTTAAGA GCACTGACTG CTCTTCCGAA GGAAGGTCCT GAGTTCAAAT 2220
CCTAGCAACC ACATGGTGGC TCACAACCAT CCATAATGAG ATCTGACACC CTCTTCTGGT 2280
GCATCTGAAG ACAGCTGCAG AGCTACAGTG TACTTAGATA TACTAATAAA TAAATCTTTT 2340
TTTAAAAAAA TGAAGAGGGA TCTGAGACAC CTCAAAAGAG ATTATGAGCA GTGACTCACG 2400
GGTGATTATC TATCCTGGAG TTTTTCCTTT CCGCTTGGCT TGCAACTGGG TGGACAGACG 2460
CCCCTTTTCA TTCACAAGAA CGGGTGCTAC ATTATTTCTG AACAAAACAG CACCTGCAGT 2520
ATGTTTACTG TCCTTGCTGA CTATGAGCAC GCGCACGCGC GCGCGCACAC ACACACACAC 2580
ACACACACAC ACACACACAC ACACACACAC ATTCAGTCTC CAGAGCTCTT GGGAAGGTCA 2640
AGAAGAGGCT GCCCTCAAAC ACGATCTTCA TCTTTCCCTC CTAAAGGAGA CCACGATTCC 2700
AAGGTGGCAG AAGATCTACA GGGGGCAGAG GCAGGGAGGG GGAAGCAGGC CATGGTTTCC 2760
AGAGACCTAC AGCAGAGGGC AGCAAGGCAG ATCCCCAGGT CCAGGGCAGG GAGGTGGAGG 2820
CCCTTGTTCC GAGGAGAAGG CAGGCGGCAG AACAGGGTTC AAAGGCACAG GTTTATGGCA 2880
GCTCATAAAA GTGGAGGTCG TGGCTCACTC AGAAAGGAGG AAGAAGGGAA AGGCCCTTGT 2940
GCCCACTGAG CGAGGGTCAT GCTGAGTAGG AGAGATCTGC AGGGGTGCCA GGAGCCCCAC 3000
CTGTCTGTCC CAAGGGAACC CCAAGTGTGA ACTCTGGCCT TGGGTGCTGA GTTCCAGCTA 3060
CAAGACCCCA GGAGTCCTAC TCCATCCCCA TCCAGTGCCC CCTCGCCCCG CCACACCCCA 3120
CCCCCGACTC CCGTGCCACT TCTCTAGGGC TGGAGGGTGG CCAGCCCTGG TGGGGGTTGC 3180
CTACCTGCAG GTAGAGCCCA GGTCCTAGCC GGAAGTGCAC CCCATCCCTG AAGCTGCAGA 3240
GCCAAGGGCG GGGCACACGG CAGCTCAGGC TGTCAGGCTG TTGCTGGGCT CTAGGTTCCC 3300
AGGGACCTGG GCACCTACTT CCCCACCCCC CCATCCATTC TCTCTGGGGC CCTATCTTCC 3360
CTTATATGGT GAAGGAAGTT CCTGGGGGGG GGGGGTGGTG GTGAGGACAA AGGTCGTTCG 3420
GTCTCCTGCA GCCAGCTTGC CACAACTTCC TAAGATCTCC CAGGTGGTGG CTGCCTCTTC 3480

+1 **exon 1**

(transcription start site)

CAGACAGGTA AGGCAATTGG GTGGGGACAC ATGGTGACCA CAGGTGGTTG GAGGGGACAG 3540
GGTCCTTGCT TCTCTCTGGC AGCCTGTGCT TTCTGTAGCA CCTTGGTATA AGTTTGGGGG 3600

FIGURE 6B

TGAGGTAAGG TGCTCTGAAA CTCTGAAAGA AGCAAGAAGC CAGCAGGCTG TCTTGGGCCT 3660
TCAATGAAGG AAGTTCACAG ACCCCCTTTC CTGTAAGTCA CCTTCGCTTC ATCTGTGTAG 3720
ATTCCCTGGG ACCAAGGTGG CTCCTGGGAC TCAGATTTCT ACAATTAAAA TCAGGACAGT 3780
CCTGAGACTT GGACTCCGTG CCTGTATTTA CTACTTCTCT CTGGCTGCTC ATTTCTGTGT 3840
TCATGTCTTA CACATCTGAA ATGGTTTCTT TGTGTCACCA TTCCCCTGAC ACTCCTGGGA 3900
GGTCGTATCC TTGGCACATG TATCCTGGGA TGTAAGCTGC AGCCACCAGG AGAGAGGGGG 3960
AGAGTCAGGA GCTGTGTCCT AGGCCCTATT AGGCCTGGAC ATCACCCCTT TCCTAGAAAT 4020
GGCCCCTCCA TTTTTCGGTT ACCATGATCT ATTTTATATC AGAGTGGGCA GTGAAAGCCA 4080
AACCTGCCCA GAAGTTTGGG ACTCACTCAG ACCAAGGTTA TCTGCTCAGA AATCCCCCTG 4140
TCACTTGAGG TTGGGAGAAT CTGCCTCTGG GGGCTTCCAG GTCTTGGTTA GCAGGAGGGT 4200
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AGACAGTAGA TATGCAAGGA TAGGTAGCTG GGGAGAAGAA GAACTTAAAC CCCCCCAAAG 4380
GCCCACAGGT TCCGTTCCCT AGTTCACAAT GCCAGTATGA GTGCTAGCTA CTATGGGCTG 4440
TGAGTTGGTA GCTACAAGCA TGAGTGATGT TCATGTGTGT AGTGTGTATA ATCTGAGCAC 4500
TTGGGAGGCT GAAGCAGGAG GATTGCTATA TGTTTGAGGC CAGCCTGAGC TATAGAGCGA 4560
GACTTTGTCT TTAAGAAAAA AATGAAAGCC CAGCAGTGGT GGCACACGCC TTTAATCCCA 4620
GCACTTGGGA GGCAGAAGCA GGCAGATTTC TGAGTTCAAG GCCAGCCTGG TCTATAGAGT 4680
GAGTTCCAGG ACAGCCAGGG CTACACAGAG AAACCCTGTT TTGAAAAACC AGAAAAACAA 4740
AACAAAACAA AACAAAACAA AACCCAAACC CAAACCCAAA CCTCTCATCT CTCATCTCTC 4800
TAGGCTGTGT CTGTCTAGGT GGTAGAGTTT GGGGACTTCA GACTTATATA TAAATAGGCC 4860
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AAGTACTCCT TGCCAGCCCA AAAATTCTGG GAAGGCTTCC TGGAGGAAGT GTGTCCCGAT 4980
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GGAACAGAAG GACAGGAGGG GGAGGCATCC AAGATTCTGA ACATGTAGCT GACTTTTGGT 5100
TCTCTGGGTG ACAAGTGTCC CCCAGGGATA GGGCTGTAGA AAGGGGACCA GGGGTGAGCC 5160
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GGGTGAAGGA GAGGTATCTG CTCTGACCAA CATGGCTAGG AGCAGAAGCA GTTGGACCAG 5340
TTACCCCTCA GAACCAGCCA TCCCCTCTTG GCTCTAAGGA GGCTGGGCCC CTTTCTGTTT 5400
AAGAATCTTA CTTTTCTTCA GAGAGAGGCA GCAAGCCTTT GTCCCCTCCC TGTTGGTCAA 5460
TAAACACCCC TGTGTGTAAC ATTAGTTTAT TTTACTGTCA GTTTGCTCCA GGACAGTCCA 5520

FIGURE 6C

TCTGGTAGAC CTCTGCTCCT AACTCACCAA GGTATGGCCC ACATTCCTCA CCCAGAAGAG 5580
TGCAGAAGAG AGCCTTAGAG AAAGGGTAAC AGTAACAAAG ATGGCCAGAA TAAAACAAAA 5640
ACTACTATCC TTTGTACCCA AATTGGTTTT GCTGAACCAG GAGGGGGTGT GTGAGTGTAT 5700
GTGTGTGTGT GTGTGTGTGT GTGTGTGTGT GTGTGTGTGT GTGTGTGTGT GTGTGTGTGT 5760
CTTGGGGGAC TTTTCATGCT AAAGAATATC TGATATTGGC GCCCATGCCA ACAGGGGTAT 5820
TGGGGAGAGT CAGGCTTCTG CAAACACAGT AAGCTGCCCA AGATGGATTG GTGGCCTGAA 5880
TCACCAAGGG GCAGGCTGAT CAGAGTGGAC AGAACATCAC AAGATAAGCC ACCCTGTGGG 5940
GCTCAGAAGA GGGAGTTTAC AAGAGGTAAA GGCCAAGCCA TTTATTATCC AAGACATGAC 6000
TCAAAATCAA AGTGCAAGGA GAGATTAGCT GGAGAGATGG GGCTGTCAGT GTGGGACACC 6060
TGACCTTGCA CTTATTAGTC ACTAGGCCAA GGAGCAGTCA CAGAGGGTGA CTGGGTCCTA 6120
CTCAGCTTGG AGCAGGCACG TGGAGAATGG GTGACCTCCA TCCTGATGGA GAGGGCTGAG 6180
CACCACCAGG TACAAGTGTT CCCTGTGTCT CATGCCAGGA TTCCTGGCCA GTTTTCAAAG 6240
GACTAAGGAC TCATCTCTGG TGGAAACAAA GTATCCAAGC CCTAAGCCCC ATTTTGGTCT 6300
AATTAAATCA GAACCCCTGG GGATGCAGGC TCTGAGCAGC AGGAGCTTTT TAAAAAGCTC 6360
CCAGGTGATT CTGATCAGCA GCTGGAACAA ACACAGCTAC AGGTTCAAAC AGAAAGAGGC 6420
AAAGCTAGGG AAAGCTTGGG ATGGGGAGCC TTCTTCCAGG CCAGTAGATG GAGGCTGGTT 6480
AGCAGTGGTG GCAGCTTCTC TCTGCCTGTC ATATAGCTAT CCATCCACTC ATCCATCCAT 6540
ACACCCACCC ATCCATTTAT GCACCCATCC TTCCATCCAT CCATCTATCC AGCTACCCAC 6600
CCACGCATCC ATCCAAACCT TCCTTTTCTC CTTCTTTCTT TCTTTTTTCC TTCACTCATT 6660
CATTTATCCA ACAGAGAACT GGTATTGTAC TAAATGTGGG AGATTTAATT AATTTTTAGA 6720
AGCTCTGTTG ATTGACTGAT TGTGCATGTA TGTGGACAGG TACATACCAC AGCACACGTG 6780
TGGCAATCGG AGAAAGGTTT TGGGTGTTGT TTTCTCTTCC CACCGTGTGG GTTCTGGGGA 6840
TTGAACTCAA ATTATCGGGC TGGTGGCAAG TGTCTTTACC ACCGAGCCAT TTTGCTGACA 6900
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CCACGGATGA CCTTTAACTC CTGCTCTTCC AGCCTCCACC CGAGTGCTAG GTTTACAGGT 7020
GTTCAACTGG TGAATGCCTT TAATCCCAGC ACTCTGTGGG GGGGGGGGGG GAGGCGGATC 7080
CCTGAGTTGG AGGCCAGTTT GGTCTACAGA GTTTCAGGAT ACCTGGGGCT ATACAGGGAA 7140
ACCCTATCCC AAACAAACAA ACAAACAAAC AAAAAATATT CTGTGCAATA ATCACAGAGA 7200
TTAGAGGATA TTAGTAGGGT AGTAGGGCTG GTGAGGGAGA GTCATGCTTT CTTTTGTATT 7260
ATAATAGTAA AGTACTCACA AGATGCATTA TCTATCTATC TATCTATCTA TCTATCTATC 7320
TATCTATCTA TCTACCTACC TACCTACCTA TCCATCCATC CATCTATCGT ATAGCCCAGG 7380
CTGCTTTGAC TCTGAATGCT CCTATTTCTG GGTCAACTCT TCACCCCTAG TGTTGGGTTT 7440

FIGURE 6D

ACCAACACCC AGACATTTAT TTTATTTTGT TTTATTTTAT TAATCTAGGA GCTCAGGGTG 7500
GGACTCAGGG TCTTGTGCAT GCTAAGCAAG CTCTCTGCCA CAGAGCTGCA GCTCCAGTCC 7560
CCATTTTGTT CAGGTGACTC TGTGACAGTT GTCATATTCG CAGCGCTATG TAGCTCTCTC 7620
CACCTCCCAG TTCCAGCACT TTCTGGTCAT CCCAGTGGGC GGGCAACTCT GTGCTCACCA 7680
GTGCCCTGTT CCCTGTCTTC AGACCTACAT ATTTGCCTGT CTGAACAGTT CATGTAAATG 7740
GGATGCGTTC CTGTGTATTC TTTTATGGCT GGCCCCTTTA TCTTAGCACA GTTTGTGTTG 7800
GGCCATGTGT CACTGCTATA CTCTATCTTA TCATCATCTT ATGGCTTAAT AGTGTTCCTT 7860
TGTGTGGATA AACCACTTTC TGTTTCATTT ACTGATGGAA ATTTGTGGCC CCACCCCCAC 7920
CCTTTTTTTT TTTATTTGAG ACAAGGTCTT TCTGTGTAAT CTTGCAATCT TGGCTGTCCT 7980
GAGCTCACTC TGTAGACCAG GCTGTGAGGC TGTCCTTCCA CTTTTGACAC TCCTGTGAAC 8040
AGAGTAGCCA TGAACTTCAA AGACAATTTT CTGTTTTGGT TTGTTTTTTA CATTTGTGTG 8100
TGTATGCGTG TATATGTGCA TGTTTGTGTC TTCAGGTGCT CACATGTGTG TACCTGTGTG 8160
TGGGACAGAG AACAAACCGA TGTGCCATTC CTCAGATACT ACGCATCTTG TTAATATGTA 8220
TGTATTATGT ATGTTTATTT AGTGTGCCCA AGTATGCAGG TATTTTGTTG GAGTTTTCAC 8280
CTTCCCTTGT GGGCTCTCCG CATTAAACTC AGCTCCTCGG GCTAGTGAGC AATGCCTTCA 8340
CTCGATGAGC CATCTCGCTG CCCCTGCTGC CACCTCCTCC TTATTTCCCA GATGGGACTA 8400
CGCACTGCAC TGGCCTAAAG CTCACCAAGT CATCCAGAGT GGCTAGCCAG GGAGACTCAG 8460
GGATATGCTG GCCTCTGCCT CCACAGTGCT AGAATTACAG GCATACATCA CTGCTGGAAG 8520
ATTTTTAACC TGAATCCTGA GGATAGAGCA GGCACTCTAC CAATGGAGGG TTCTTTTTGT 8580
GTTTGGTTTG GTTTCCTCTG CATAAGATCA GGCAGTCTGA AATAGTGTAG CCTGGGCTAC 8640
ATAACATCTT GTCTCAAAAA GCCTATAGAG GTAGGGAGGT CGAGGCTAAA GAAGAGCCTT 8700
AAGCCGGCTG TGATAGCACA CAGGATAGCC TGCACTATAT AGCAAGACCT TGTTTCAAAA 8760
ACATGGAGGG AGGGGTATGT TTTAAGTGCT GGGCTGTGTA ACAGGCACTA AGGGAGCCAA 8820
TGTAGACATT TGACTAAGAA AGGATCATCA TCAAAGCCGG GTGGGCAGGG TAGAGGTTGG 8880
ACTACAGTGG TCAAGACCCC CATAGGAAGC CAGTTTCCCT TCTTCCTCTG GGCCTCAAGC 8940
CTGGCTCGAC GGCCACTGCT CTCACATGCC TTCTCCTCTA GGCTCGTCCA CCATG 8995

exon 2

FIGURE 6E

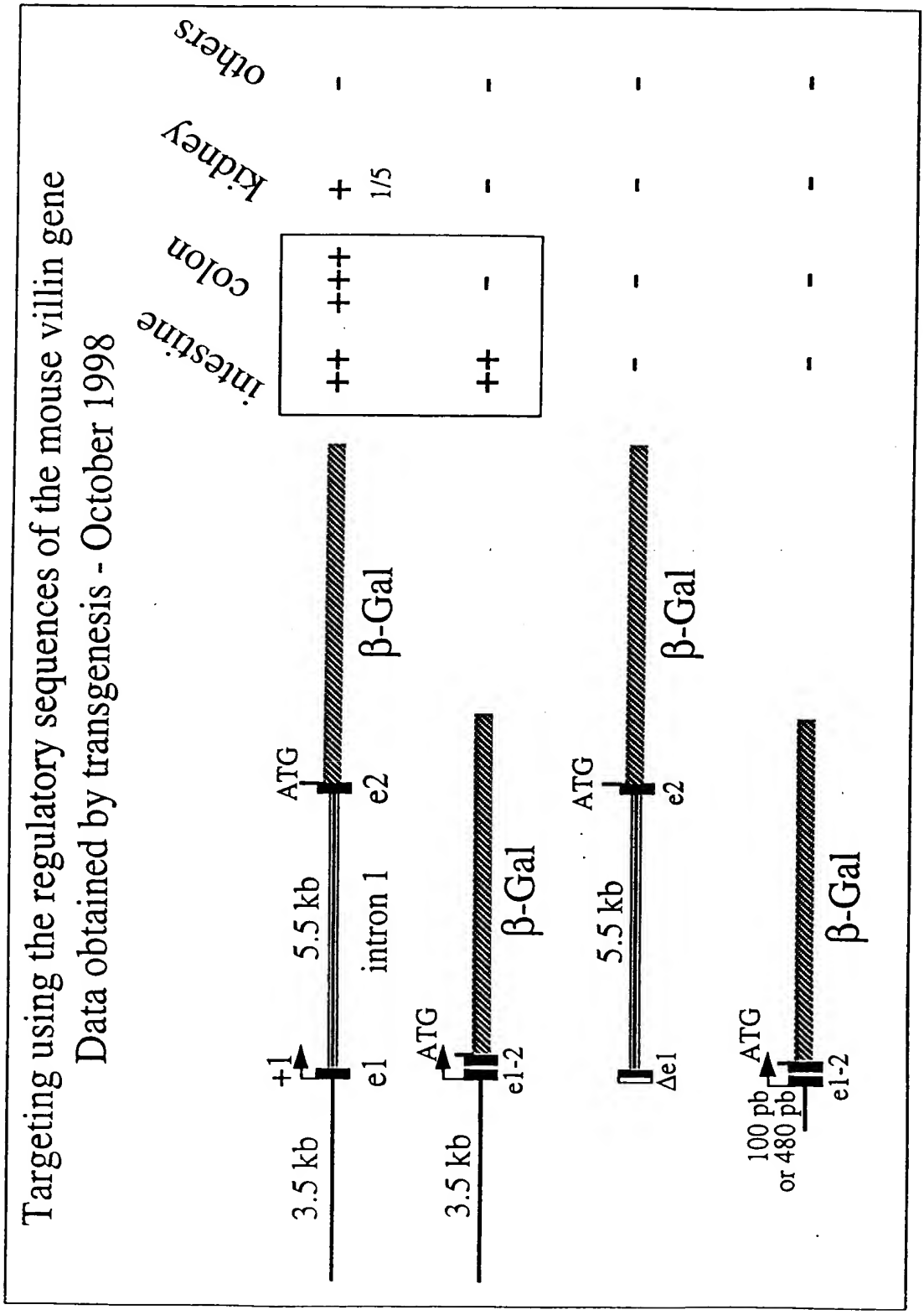
Targeting using the regulatory sequences of the mouse villin gene
Data obtained by transgenesis - October 1998
intestine
colon
kidney
others
++
+++
+
-
1/5
3.5 kb
+1
e1
5.5 kb
intron 1
ATG
e2
β-Gal
3.5 kb
ATG
e1-2
β-Gal
5.5 kb
ATG
e2
β-Gal
Δe1
100 pb
or 480 pb
ATG
e1-2
β-Gal

Figure 7

Targeting of oncogenes and tumor suppressor genes

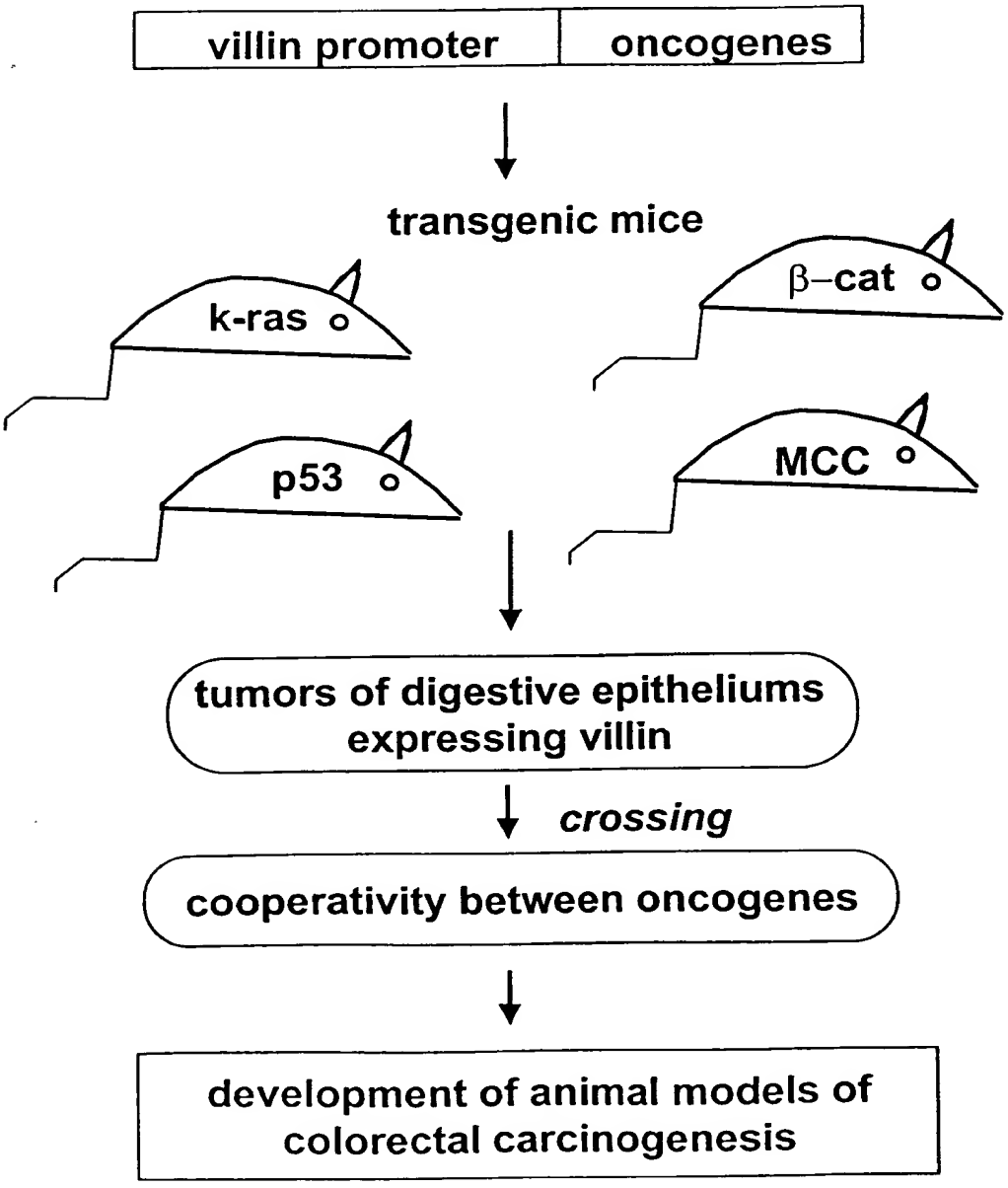

FIGURE 8A

Targeting of immortalizing gene

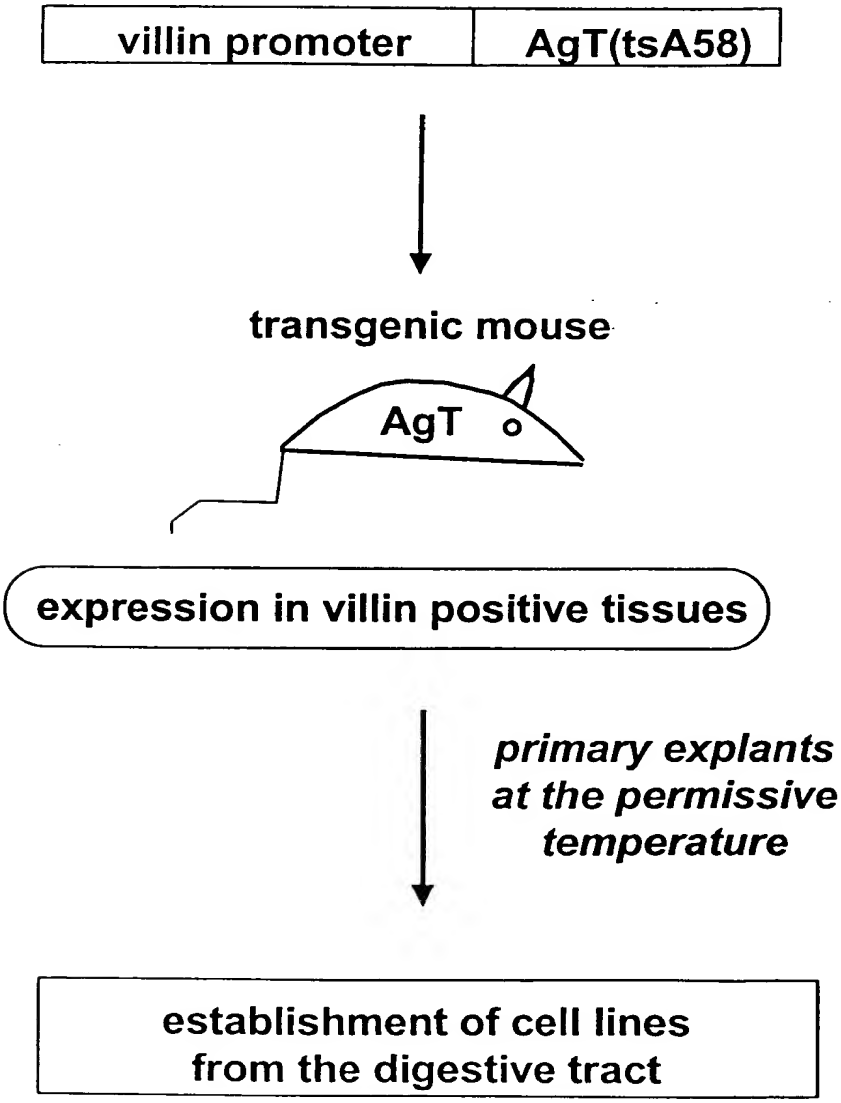

FIGURE 8B

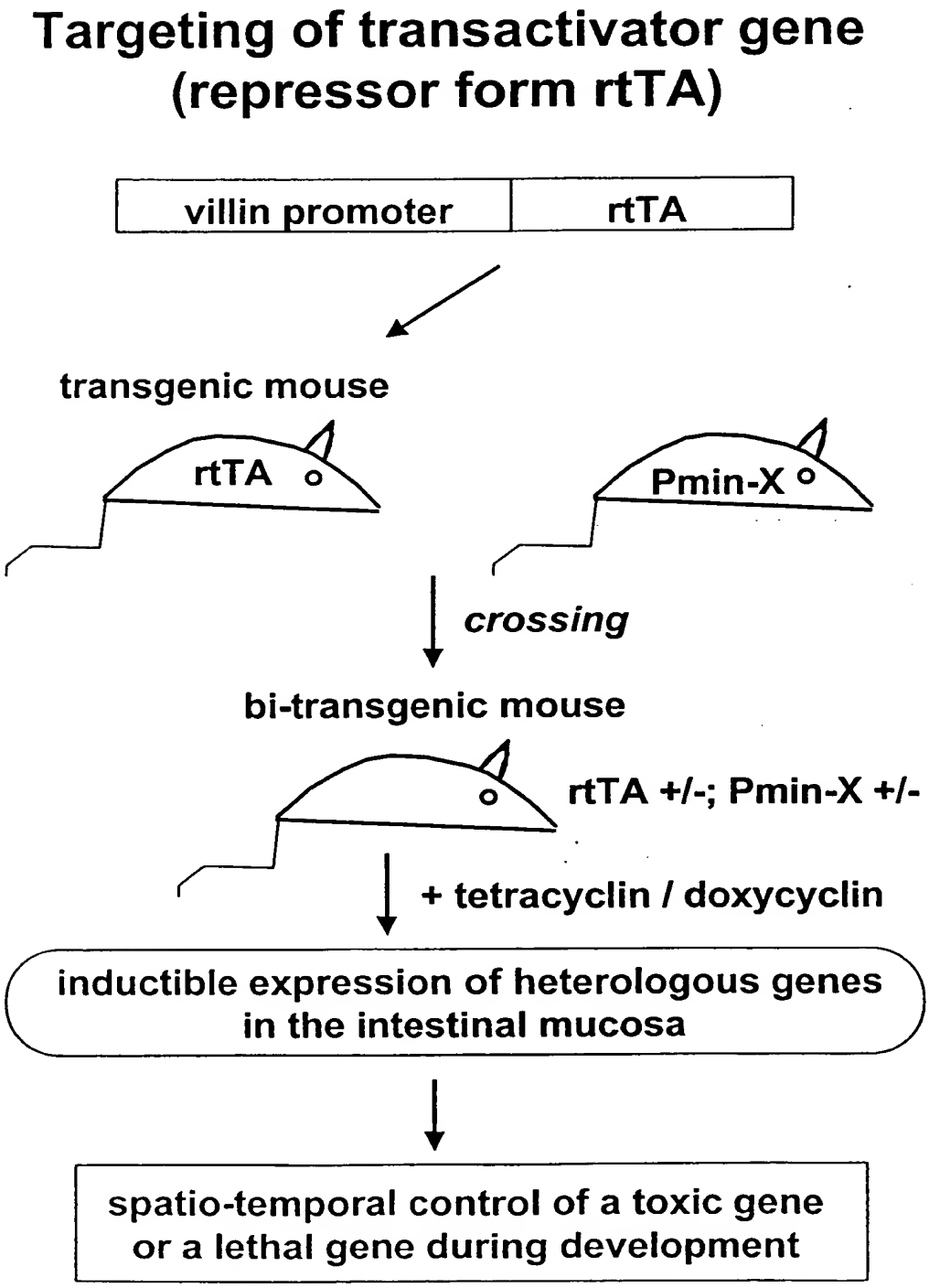
Targeting of transactivator gene (repressor form rtTA)
villin promoter
rtTA
transgenic mouse
rtTA
Pmin-X
crossing
bi-transgenic mouse
rtTA +/-; Pmin-X +/-
+ tetracyclin / doxycyclin
inductible expression of heterologous genes in the intestinal mucosa
spatio-temporal control of a toxic gene or a lethal gene during development

FIGURE 8C

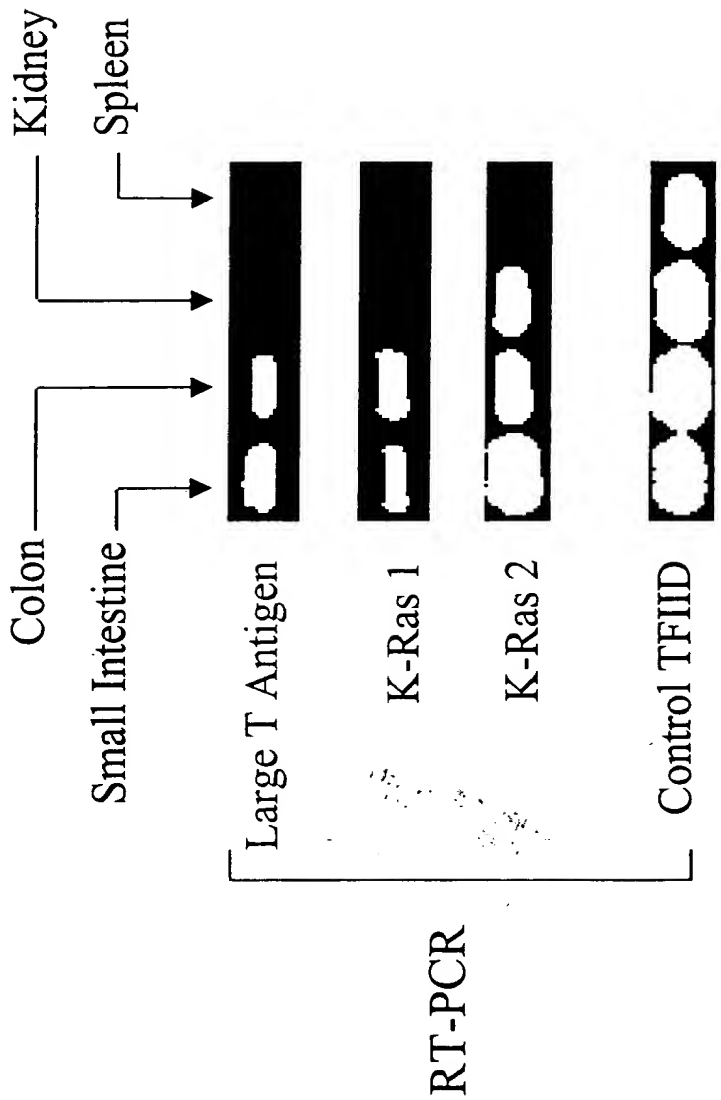
Kidney
Spleen
Colon
Small Intestine
Large T Antigen
K-Ras 1
K-Ras 2
Control TFIID
RT-PCR

FIGURE 9